The Influence of MED 12 Knockdown on Adipogenesis

Jamie Sparkman[1], Emily Meaney[1], Sree Venigallat[1], Joseph Straub[1], Jamie Newman[1]

[1]School of Biological Sciences, Louisiana Tech University, Ruston, LA 71272 [2]Biomedical Engineering, Louisiana Tech University, Ruston, LA 71272

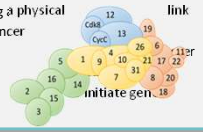
Objective: Determine the effect on gene expression on different subunits of the kinase module after MED 12 has been knocked down.

Background

Stem cells are cells that retain the capacity to self-renew and differentiate into many different cell lineages. Human adipose-derived stem cells (hASCs) are stem cells that have been isolated from adult fat tissues. hASCs are multipotent and regenerative. These traits, combined with non-invasive collection methods, make hADSCs favorable in the search for new clinical stem cell treatments - such as tissue regeneration and repair. Adipogenesis is the process in which stem cells (pre-adipocytes) differentiate into adipocytes. While the mechanisms behind differentiation are not fully understood, it is known that transcription factors control the pathways of differentiation (Mariani, 2004).

Transcription factors are proteins that are involved in activating or inhibiting transcription and gene expression. The Mediator complex is a large RNA polymerase II transcriptional regulator that is divided into four distinct modules: the head, tail, middle, and CDK8 kinase (Figure 1). The Mediator is known to serve a key role in the activation and repression of transcription and believed to position genes in nuclear space. MED12 is found in the CDK8 kinase module subunit along with MED13, CDK8, and CCNC.

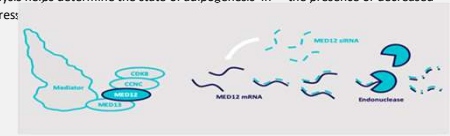
The Mediator complex is a group of thirty subunits that function together to regulate gene expression . It does this by creating a physical link between the transcription factors bound at enhancer elements and RNA polymerase II bound at the region. When brought together by factors activate RNA polymerase II transcription (Medline).



Project Overview

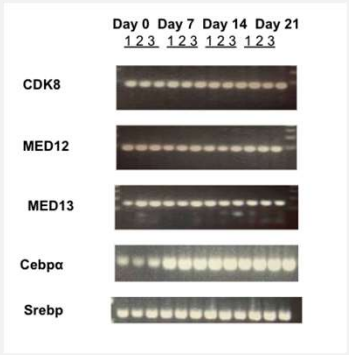
We are interested in understanding the function of MED 12 in adipogenesis and determine its role in initiating cell types specific gene expression. MED 12 is a subunit of the Mediator complex kinase module. If we understand the mechanisms involved in regulating cell type-specific gene expression, then we can manipulate stem cell fate, which will benefit the further application and knowledge of stem cell therapies available. To analyze the role that MED12 has in adipogenesis, we first have to determine how adipogenesis is affected when MED12 is absent or has been knocked down in these cells.

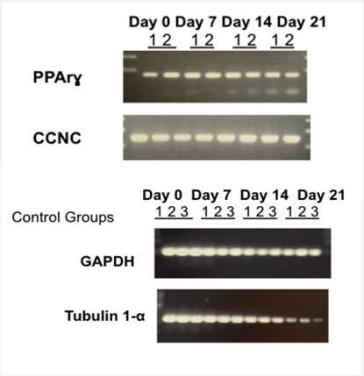
We will first culture hASCs in media until they have reached 60% confluency, at which point the cells are transfected with MED12 siRNA or negative control.24 hours after transfection, adipogenesis is induced and the cells are cultured again but now in adipogenic media s. RNA is then extracted and quantified from the samples on Days 3,7, and 14 after the induction of adipogenesis and then utilized to make cDNA and run polymerase chain reaction (RT-PCR) to occur. This information is then used to compare and analyze the samples where MED12 is present to those where MED12 was knocked down to see if adipogenesis is occurring. To validate the knockdown we expect to see a significantly lower expression of MED12 in the knockdown samples compared to the negative control. In order to see if adipogenesis is occurring, the expression level of PPARγ is utilized. PPARγ is an adipogenic marker, therefore, its expression will increase when adipogenesis has occurred. This comparison and analysis helps determine the state of adipogenesis in the presence or decreased expres



Identifying Expressions of Kinase Subunits and Effect of Knockdown

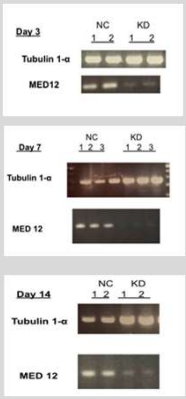
Objective 1: MED12 and Kinase Subunit Expressions





- These gel images serve to visualize gene expression levels of all different kinase modules at different time points.
- The different time points included here are day 0, day 3, day 7, and day 14.
- The increase in expression in days supports that inducing adipogenesis early will ensure that fat cells are able to fully develop and work properly.

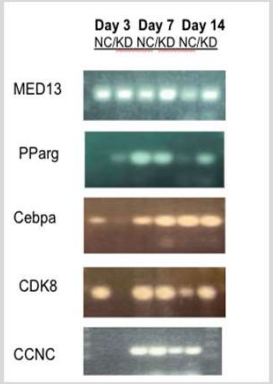
Objective 2: Knockdown of MED12



Med 12 has varied functions in different types of cells, making it an appropriate candidate to knockdown to show that it is important in the kinase module.

- These images taken of gel include samples of Med 12, the gene of interest, and tubulin 1-alpha, used as a loading control.
- These serve to validate the knockdown of Med12.
- The gene expression shown in the negative control samples of Med 12 compared to the little to no expression of the knockdown samples accomplishes the purpose.

Objective 3: Observing the Effect of MED12 Knockdown



MED13 was unaffected.

- This is because this subunit can bind to the Core Mediator Complex in the absence of MED 12 and other kinase subunits.

CDK 8 was affected in day 14 expression.Cyclin C was affected on day 14, and showed slightly less expression.

- This is due to the fact that these two subunits must bind to MED 12 in order to ultimately bind to the mediator complex.

PPARγ and CEbpα both easily showed less expression meaning less adipogenesis is occuring. This also proves that there is a relationship between these adipogenic initatories.

Future Applications

1. Further Investigate Protein-Protein Interactions

In future research, we would like to explore the mechanisms behind protein-protein interactions between MED12 and adipogenic regulators such as PPARγ and cebpα. Identifying a relationship between MED12 and PPARγ and cebpα would allow a better understanding of how the expression of these proteins affect adipogenesis.

- PPARγ is considered a master regulator of adipogenesis (Wafer).
- Examining cebpα through Wester Blotting shows the interaction between transcription factors (proteins) and MED12.

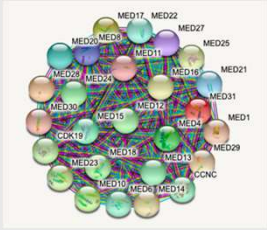


Figure 1: This figure serves the purpose of visualizing the protein-protein interactions between kinase subunits.

2. Knocking down other Kinase Subunits and Observing the Effect

Furthering this research, we plan on looking deeper into the role other kinase subunits play and at what point are they important in lipogenesis.

- CDK8 and Cyclin C are known regulators of lipogenesis (Zhao).
- With that being known, knocking down the CDK8 and Cyclin C genes, and observing the effect/expression of other kinase subunits within the Mediator complex could provide more reasoning and explanation behind the specific role in lipogenesis that they uphold.

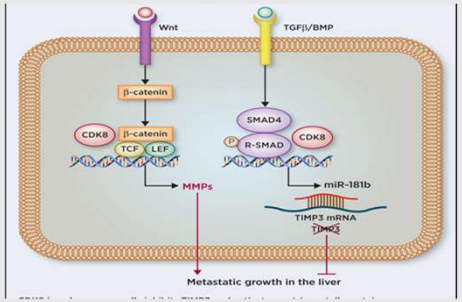


Figure 2: Lipogenesis occuring in a colon cell. This figure identified the genes, specifically CDK8, that play a role in this process (Liang).

References

Mariani, A., Bidali, S., Fiori, S., Malucelli, G. and Ricco, L. (2004), New Vistas in Frontal Polymerization. 1

Wafer R, Tandon P and Minchin JEN (2017) The Role of Peroxisome Proliferator-Activated Receptor Gamma (PPARG) in Adipogenesis: Applying Knowledge from the Fish Aquaculture Industry to Biomedical Research.

Zhao, X, Fen D. (2012, July 02) Regulation of lipogenesis By Cyclin-Dependent KINASE 8-mediated control of SREBP-1.

Liang, J., Chen, M., Hughes, D.. (2018, December 01). CDK8 selectively promotes the growth of colon Cancer metastases in the liver by regulating gene expression Of TIMP3

Acknowledgements

We would like to thank the College of Applied and Natural Sciences of Louisiana Tech University for the mini-grants, which helped to purchase materials. We would also like to thank the LBRN program for providing access to iPoster. Thank you to the Newman lab for providing a space to work, materials, and guidance.

NEWMAN LAB

LBRN Louisiana Biomedical Research Network